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BUTYRIC ACID BY FERMENTATION

By RAFAEL ARROYO,

Chief, Division of Chemistry, Agricultural Experiment Station of the College of Agriculture of the University of Puerto Rico, Río Peidras, P. R.

INTRODUCTION

The writer has been interested in industrial fermentation problems since 1929. On various occasions he has expressed his firm belief in the glorious future awaiting fermentation processes in the field of organic industrial chemistry. He has, moreover, expressed his belief that no other territory is better adapted to industrial fermentation work than the tropics; especially in its application to carbohydrate material. The products, by-products, and so-called waste products of the sugar-cane industry offer an abundant and cheap supply of raw material; while the microbiological flora offers in a generous measure the organisms through whose agency the transformation of carbohydrate raw material into useful chemicals will take place.

In the summer of 1931 the writer started a research for the production of butyl alcohol and acetone by a special fermentation carried on blackstraps through the agency of an organism, (B. Tetryl), discovered and isolated by him.

In March, 1934, patent application was made before the U. S. Patent Office for this process, and patent rights were granted by the Examiner in April 1935. English patent application is now pending.

A detailed account of the laboratory work connected with this research was published in the October 1934 number of "The Journal of Agriculture of the University of Puerto Rico", which was issued on December of the same year.

In the second part of that publication, mention was made of another investigation started during 1933 along similar lines; this time the work aiming at the production of N. Butyric Acid from blackstrap molasses. The purpose of this paper is to give a full account of the results of this new investigation.

The writer's intention being to apply for patent rights protection for this new process, and eventually enter into its commercial exploitation, he has already presented a petition to the "Board of Trustees of the University of Puerto Rico" to grant him their consent and approval of this action.

Butyric Acid is a Valuable and Important Acid

Butyric acid is one of the most valuable aliphatic acids used commercially; its main drawback to an extended use in the chemical industries being its unnecessary and inexplicable high price. In striking contrast with the declining values that were prevalent in the New York Chemical Market from the latter part of 1929 to the first half of 1934, the price of N. Butyric Acid remained practically constant at 80 cents per pound, based on hundred-percent acid. Recently the introduction of synthetic butyric acid by the Carbon and Carbide Corporation has had a detrimental effect on the price of this commodity; but even so, the price remains too high to encourage a carload use for this chemical. With a view to find a process that would make it possible to sell N. Butyric Acid, with a profit, at a price less than its present cost, this investigation was effected. The success encountered in our previous investigation on the production of butanol and acetone by fermentation, encouraged us to try similar methods in this new study.

Difficulties Encountered by Previous Investigators

Although processes leading to the production of organic acids by fermentation have been practiced from time inmemorial, the mechanism whereby these processes took place, or the agents responsible for them, were utterly ignored. The production of butyric acid remained in the situation common to all these processes until Pasteur recognized the butyric fermentation as a well defined microbiological phenomenon; describing this fermentation as an anaerobic process before the Academy of Science in Paris, 1861.

Since this date, many groups of these organisms have been found and their products of fermentation studied by several well-known investigators; among which we may mention Kirov; Baier; Fitz; Winogradsky; Buchner and Meisenheimer. These men of science agreed that the formation of butyric acid by fermentation when using the organisms known to them, was accompanied by secondary reactions, producers of a variety of other substances. For instance, Buchner and Meisenheimer when working with the "Bacillus

Butyricus'' Fitz, found the following products as typical of the fermentation of 100 grams glucose: 0.7 grams butanol; 2.8 grams ethanol; 1.6 grams hydrogen; 3.4 grams formic acid; 10 grams lactic acid; 7.5 grams acetic acid; and 26 grams butyric acid.

Recently, the chemists H. T. Herrick and O. E. May of the Department of Agriculture, Washington, D. C., published a circular on the production of organic acids by fermentation in which they opined that the butyric fermentation had not been applied industrially in commercial magnitude due to the great variety of substances other than butyric acid produced during the fermentation.

The patent literature describes some processes which give the impression that the work has been done in decidedly empirical form. In some cases it is really difficult to understand how a patent could be secured on such vague, indefinite and entirely unscientific data.

Attacking the Problem

Having acquired from the literature a knowledge of the butyric fermentation whose synopsis is given above, it was resolved to attack the problem of butyric-acid production from waste or final sugar-factory molasses using a native bacillus found by the writer. The work was started during July 1933.

Procedure

The laboratory work outlined to carry out this investigation consisted in the first place in the preparation of a suitable medium (using blackstrap as raw material) for the propagation and development of the bacteria, and the production of the desired product of fermentation; and in the second place, the determination of the most favorable conditions of carrying this fermentation, leading to maximum yields with minimum expenditures. This meant the preparation of a ''Standard Mash'' with such physiochemical modifications as would render it the most suitable menstruum for this particular type of bacillus; and the running of fermentation tests to determine:

(1) *Ability of the Bacillus to Attack both Sucrose and Invert-Sugar for Acid production; or its Hydrolytic Power to convert Sucrose into Invert-Sugar, and then these simple Sugars into Acid.*

This first step was very important since blackstrap consists essentially of a mixture of sucrose and invert-sugar in the approximate ratio of 2:1 respectively. The result of the experiment would determine the necessity of inverting the sucrose content of the mo-

lasses, prior to mashing; a process simple enough in the laboratory scale; but that often brings great difficulties of a chemical, economic, and mechanical nature when the same simple reaction is tried in a manufacturing magnitude.

(2) *Optimum pH Value.*—In industrial fermentation, as well as in all biological processes, the adjustment of the reaction of the nutrient medium is of the utmost importance. A change in the pH value may vary the end products of microbial metabolism in type and in percentage. Besides, there exists limits in which microbial growth is possible, and each and every group of microorganisms has a definite optimum hydrogen ion concentration for its metabolism.

(3) *Optimum Fermentation Temperature.*—The external temperature is related to the metabolism of the microbial cell; especially so, due to the poikilothermic nature of bacteria. The external temperature is adjusted in relation to the specific fermentation, keeping in mind that the optimum temperature for growth may not necessarily be the optimum for the production of specific end products. It would be well to mention in this connection that the particular strain of butyric ferment used in this work differed in a marked degree in respect to optimum temperature of fermentation from most others that have been mentioned in the literature; the generally accepted optimum being between 35 and 38 deg. C. while in our case we found 30 to 32 deg. C. to be the optimum.

(4) *Optimum Sugar Concentration.*—The importance of this determination is self-evident. The higher the sugar concentration that may be carried during mashing operations compatible with high yields, and ease of fermentation, the better economic results may be obtained when the process is commercially exploited. It means higher unit yields; and economy of equipment, space, power and fuel. In one word: higher net profits.

(5) *Use of Activators.*—It is well known that there are certain substances that help the fermenting organisms in their work, shortening the duration of fermentation or increasing the yield; or acting in both of these directions at the same time. Among these, we may mention various forms of carbon such as charcoal, lamp-black, bone-black, activated vegetable chars, as Darco D–4; diatomaceous earth; various inorganic salts as sodium chloride, manganous sulphate, etc., etc.

In his previous investigation for the production of butanol and acetone, the writer used some of the above-mentioned substances with great success. As will be shown later on, in the present case

these activators had no beneficial effect whatsoever when applied to the butyric acid fermentation.

(6) *Effect of Substituting Glass Fermenters for Others Made of Different Materials.*—This experiment was of great importance from a technical and economic standpoint; since, when transferring the laboratory experimental work to pilot plant operations, glass would hardly be the most adequate material of construction.

(7) *Effect of Surface—Volume Ratio.*—Another important point of consideration; for sometimes a great variation in yield follows from modifications of this ratio.

(8) *Determination of the Advantages of Incorporating Additional Nitrogenous Nutrient to the Mashes Besides Those Found Naturally in the Raw Material.*—Blackstrap contains most of the necessary nutrients for microbial growth; its deficiency in this respect being in most cases in nitrogen. The nitrogen content of blackstrap varies considerably with the country where it is produced and appreciably with different localities in the same country. As extremes, may be taken Cuban and Egyptian molasses; the former running on an average of 1.00 to 1.25 percent nitrogen, while the latter runs as low as 0.3 percent and very seldom above 0.5 percent. Puerto Rican molasses runs on the average of 0.65 per cent nitrogen. It was, therefore, found advisable to determine the effect upon fermentation of the addition of a cheap source of inorganic nitrogen, as sulphate of ammonia. As will be seen later on, the results of this experiment were very interesting.

The experimental data in tabular form (with pertinent comments thereon) in connection with the different topics outlined above, will now follow:

(1) *Determination of the ability of the bacillus to use both sucrose and invert-sugar for acid production; or its hydrolytic power to convert sucrose into invert-sugar and then these simple sugars into acid.*

A set of six mashes was prepared using various mixtures of sucrose and invert-sugar, varying from 100 percent sucrose to 100 percent invert-sugar content. Pure sucrose, laevulose and dextrose were used in the experiment, the mashes being artificially prepared with the addition of blackstrap molasses-ash, and nitrogenous nutrients for the bacteria. The reason to use various mixtures of sucrose and invert-sugar, besides these sugars separately, was due to the belief that while the organism might not be able to attack sucrose in a pure sucrose medium, it might be able to do so when the sucrose was mixed with invert-sugar. The different mashes were autoclaved

for a half hour at 15 lbs. G. P.; cooled down to room-temperature, (about 28° C.) and inoculated with a pure culture of B. Butyricus.

At definite intervals during the fermentation samples were withdrawn from individual mashes and analyzed for residual sugars.

The results will be found in the table below:

TABLE No. I

Mash No.	Total Sugar Content at Setting Time		Total Sugar Content after 36 Hours Fermentation		Total Sugar Content after 72 Hours Fermentation		Total Sugar Content on Completion of Fermentation		Completion of Fermentation Hours
	Sucrose %	Invert Sugar %	Sucrose %	Invert Sugar %	Sucrose %	Invert Sugar %	Sucrose %	Invert Sugar %	
1.....	4.50	0.00	3.00	0.50	1.50	0.75	Nil.....	0.45	120
2.....	3.00	1.50	1.55	0 70	0.70	0.60	Nil.....	0.16	96
3.....	2.00	2.50	0.85	1.35	0.32	0.64	Nil.....	0.21	96
4.....	1.00	3.50	0.78	1.40	0.15	0.87	Nil.....	0.23	92
5.....	0.50	4.00	0.29	2.00	0.00	1.02	Nil.....	0.25	92
6.....	0.00	4.50	0.00	2.05	0.00	0.98	Nil.....	0.18	89

The data in Table No. 1 shows in a definite manner that previous inversion of the sucrose content of the molasses became unnecessary, since the B. Butyricus showed in this test its ability to attack both sucrose and invert sugars during fermentation. It shows, moreover, that the bacteria would work at its best in a mixture of these sugars, or in invert-sugars alone, as regards the duration of fermentation. In the case where only sucrose was found in the medium, the bacillus took 24 additional hours to complete the fermentation; leaving besides a higher percentage of residual sugars in the fermented mash. The conclusion was arrived at that blackstrap had very good prospects of being a suitable medium since its approximate sucrose invert-sugar ratio agrees quite closely with conditions represented in mash No. 2. From a technical and economic viewpoint it was very fortunate that no inversion of the sucrose in the molasses became necessary prior to mashing.

(2) *Optimum pH Value.*—The importance of this determination has been already discussed elsewhere in this paper.

A series of eleven mashes running from pH 6.0 to pH 7.0 were prepared, using blackstrap of initial pH 5.5. The pH adjustment at mashing was accomplished y the addition of different amounts of calcium carbonate; and these initial values were maintained in individual mashes during the entire period of fermentation through the use of a 100th normal sodium hydroxide solution. The total sugar concentration of all mashes was 4.5 per cent. This value for total sugar concentration was adopted from the results on Table No. 1, as it was evident that the organism could stand this concentration.

The most convenient pH value to use subsequently was to be determined from a consideration of the following items:

a. Starting time of fermentation.
b. General apparent activity during fermentation.
c. Finishing time of fermentation.
d. Residual sugar content of mashes.
e. Yield of Total Acidity.

TABLE No. 2

Mash No.	pH value	Fermentation			Residual Sugars %	Total Acidity Grams	Total Acid Yield % Total Sugars
		Time taken to start	Time taken to finish	General activity			
1.....	6.0	18 hrs.....	101 hrs....	Fair.....	0.51	1.41	31.4
2.....	6.1	18 hrs.....	101 hrs....	Fair.....	0.49	1.43	31.8
3.....	6.2	18 hrs.....	100 hrs....	Fair.....	0.43	1.47	32.7
4.....	6.3	18 hrs.....	98 hrs....	Fair.....	0.38	1.46	32.5
5.....	6.4	16 hrs.....	98 hrs....	Fair.....	0.30	1.48	32.9
6.....	6 5	16 hrs.....	96 hrs....	Good.....	0.26	1.55	34.6
7.....	6.6	14 hrs.....	96 hrs....	Good.....	0.22	1.56	34.7
8.....	6.7	14 hrs.....	96 hrs....	Good.....	0.20	1.57	34.8
9.....	6.8	12 hrs.....	90 hrs....	Very Good..	0.18	1.61	35.9
10.....	6.9	10 hrs.....	88 hrs....	Very Good..	0.16	1.61	35.9
11.....	7.0	10 hrs.....	88 hrs....	Very Good..	0.16	1.63	36.2

From a consideration of the data on Table 2, it appears that the optimum pH value was from pH 6.8 to pH 7.0. Later developments proved that the best practice was to add at once all of the calcium carbonate necessary to neutralize the acid produced during the fermentation. The actual pH values on mashes thus prepared generally ran between pH 7.0 to pH 7.2 as the initial value; these values changing to a range between pH 6.6 and pH 6.9 on completion of the fermentation.

(3) *Determination of Optimum Temperature of Fermentation.*—Although the literature on this subject stated that most butyric acid organisms worked better at from 35° to 38° C., we had carried our two previous fermentation tests at room temperature (about 28° C.) with apparent satisfactory results. This decided us to try a range of from 28° to 40° C. for determining the optimum for this particular strain.

Thirteen sets of six mashes each were prepared, and each set fermented at a different temperature from 28° to 40° C. As it would have taken too much space to report the results of the different individuals in each set, the following table has been prepared giving the average results obtained in each. All conditions of fermentation excepting the temperature factor were maintained constant for all sets.

TABLE No. 3

Mash Set No.	Ferm. Temp. °C.	Duration of Ferm. Hrs.	% Total Sugars in Mash	% Residual Sugars after Fermentation	Sugars Fermented % Total Sugars	Yield T. Acidity % on Mash	Yield T. Acidity % on T. Sugars	Yield T. Acidity % on Sugars Fermented
1.....	28	108	4.50	0.16	96.44	1.69	37.55	39.05
2.....	29	100	4.50	0.19	95.77	1.66	36.88	38.45
3.....	30	96	4.50	0.15	96.66	1.69	37.55	38.90
4.....	31	96	4.50	0.16	96.44	1.70	37.77	39.15
5.....	32	96	4.50	0.16	96.44	1.71	38.00	39.42
6.....	33	96	4.50	0.19	95.77	1.65	36.66	38.21
7.....	34	96	4.50	0.21	95.33	1.63	36.22	38.00
8.....	35	92	4.50	0.25	94.44	1.61	35.77	37.84
9.....	36	92	4.50	0.40	91.11	1.52	33.77	37.16
10.....	37	92	4.50	0.49	89 11	1.47	32.66	36.70
11.....	38	90	4.50	0.53	88.22	1.45	32.22	36.55
12.....	39	90	4.50	0.65	85.55	1.38	30.66	35.92
13.....	40	88	4.50	0.81	82.00	1.29	28.66	34.89

From a perusal of Table No. 3 it will be apparent that optimum fermentation temperature lies in the range 30°–32° C. This places our strain fully in the mesophilic range.

It is true that at 28° C. the bacillus seems to work almost as well in so far as yield is concerned, but it will be noticed that twelve additional hours are required in this case to carry fermentation to completion, and this is a fact that must be reckoned with when working in commercial scale. A few hours are gained in finishing the fermentation when working at higher temperatures, say, from 38 to 40° C.; but the comparatively low yields of total acidity more than offset this advantage. The great difference in yields is specially apparent in the column of the table under the heading "Total Acidity Yield Percent on Total Sugars"; when a difference of over 9.0 percent is found between results of Mash Set No. 5 and 13.

This ability to work at its best at rather low temperatures is characteristic of this particular strain of B. Butyricus as already pointed out, differentiating it from most other known bacteria of this genus.

(4) *Determination of Optimum Sugar Concentration.*—To find out this very important point, the following experiment was carried out:

A set of seven mashes was prepared using ascending values of sugar concentration from 4.59 to 8.52 percent. Optimum conditions previously determined were used in this test, and all factors except that of percentage sugar concentration were kept constant.

The results will be found on Table No. 4.

TABLE No. 4

Mash No.	T. Sugars Content Grams	T. Sugars %	T. Solids %	Ferment. Time Hrs.	Residual Sugars %	T. Acidity Yield Grams	T Acidity Yield % T Sugars
1.....	18.37	4.59	7.40	95	0.15	8.43	45.90
2.....	21.00	5.25	8.50	95	0.18	9.69	46.15
3.....	23.60	5.90	9.60	96	0.16	10.83	45.91
4.....	26.30	6.58	10.60	96	0.15	12.22	46.50
5.....	28.90	7.22	11.70	98	0.25	12.78	44.22
6.....	31.50	7.88	12.80	102	0.55	12.29	39.01
7.....	34.10	8.52	13.80	108	0.75	13.00	38.12

The above figures show that optimum sugar concentration lies at 6.58 percent. At this concentration the total acidity yield percent on total sugar content of mash is the highest, while the duration of fermentation is fairly good. At sugar concentrations below this, down to 4.59 percent good results are also obtainable as regards percentage yield on sugars; but it will be noticed that the absolute unit yield, i. e. grams acidity per mash distilled, is of course much higher at the optimum of 6.58 percent sugars. On the other hand, when we try to increase the sugar concentration above 6.58 percent, descending yield values are obtained, which are quite noticeable specially when using concentrations of 7.88 percent and 8.52 percent respectively. In these cases, besides, we have an increasing value in residual sugars, and in duration of fermentation. Moreover, the absolute yields are in one case lower than at optimum concentration, and in the other case the slight increase is more than offset by the much higher percentage of sugars used in the preparation of the mash, and the much higher loss in residual sugars. From the above considerations we decided to adopt 6.58 percent as the optimum value for sugar concentration.

(5) *Effect of Activators.*—The beneficial effects to be usually expected from the use of activating agents in industrial fermentation work affect either the yield or the duration of fermentation, or both of these factors. We decided to try only cheap materials, some of which we had successfully used in our previous researches on the aceto-butylic fermentation work. Those selected were: Lamp Black; Bone Black; Wood Charcoal; Darco D–4; and Infusorial Earth.

For this determination six sets of mashes were prepared, using a different activating agent in each of the first five, and the remaining one was used as a check, no activator being added. The activating substances were used in different quantities in individual members of each set, and all optimum conditions previously determined were maintained during these tests.

To avoid using too much space only the average results obtained from each experimental set are recorded in Table No. 5 below:

TABLE No. 5

Mash Set No.	Activator Used	Ferment Time Hours	Residual Sugars %	Total Yield Grams	Total Acidity Yield % Total Sugars
1	Wood Charcoal	102	0.27	11.78	44.79
2	Lamp Black	98	0.21	11.97	45.51
3	Bone Black	100	0.19	12.00	45.62
4	Darco D-4	96	0.18	12.15	46.19
5	Infusorial Earth	102	0.23	11.87	45.13
6	None	96	0.18	12.40	47.14

The results of Table No. 5 hardly need discussion. From a consideration of the figures under the columns headed "Fermentation Time" and "Total Acidity Yield Percent on Total Sugars" it is obvious that neither the duration of fermentation nor the percentage yield were increased by the use of activators. As a matter of fact, the best results were obtained when no activating substances were used.

(6) *Effect of Substituting Glass Fermenters for Others Made of Different Materials.*—For this test, fermenters made out of (1) Sheet Iron; (2) Copper; (3) Enamelled Iron; and (4) Wood, were substituted for the glass fermenters used in all the previous tests.

A set of 6 mashes was made for each kind of fermenter used; all other conditions except the material of construction of the fermenters in the different sets, being kept the same.

On table No. 6 below, will be found the average results obtained in each case.

TABLE No. 6

Mash Set No.	Kind of Fermenters Used	Total Sugars Grams	Ferment. Time Hrs	Residual Sugars Grams	Sug. Ferm % T. Sugars	Acid Prod. Grams	Acid Prod. % T. Sug.	Acid Prod. % Sug. Ferm.
1	Iron	26.30	60.00	17 60	33.46	1.93	7.33	22.15
2	Copper	26.30	*0.00	26.30	0.00	0.00	0.00	0.00
3	Enamelled Iron	26.30	96.00	0.58	97.79	12.07	45.90	46.92
4	Wood	26.30	92.00	0.42	98.40	12.15	46.18	46.94

*In the case of the copper fermenters, fermentation never started.

The above results show in a definite manner that metallic fermenters are inhibitive to the bacterial action, copper especially so. In the case of enamelled iron and wood the results are in all respects comparable with those obtained when using glass fermenters.

(7) *Effect of Surface-Volume Ratio.*—This test was of particular importance in a study of this nature, where the ultimate objective of the work is the establishment of this process in commercial magnitude should the final result warrant such action.

Six sets of mashes were prepared as usual; the only variable this time being the amount of individual mash used in each set. The sizes of mash for the different sets were selected as follows: 100 ml.; 250 ml.; 250 ml.; 500 ml.; 1000 ml.; 2000 ml.; and 4000 ml. Care was taken to add the same percentage of inoculant based on total mash volume in all cases; the percentage used being 5.

Average results obtained in each set are recorded in Table No. 7.

TABLE NO 7

Mash Set No.	Size of Mash Ml.	Fermentation Time Hours	Residual Sugars %	Total Acidity Yield % Total Sugars	Fermentation Efficiency % Theoretical Yield
1	100	120	0.26	45.15	92.33
2	250	105	0.22	45.90	93.86
3	500	96	0.19	46.40	94.88
4	1000	96	0.18	46.90	95.91
5	2000	88	0.20	47.10	96.31
6	4000	86	0.18	47.50	97.13

The results shown on table No. 7 are quite satisfactory as regards the surface volume ratio effect. As the ratio decreases, i. e. as the volume of mash is increased, it may be noticed that not only the yields of total acidity based on Total Sugars, and the Fermentation Efficiencies increase; but that also the duration of the fermentation is shortened at the same time. The results give us reasonable assurance that in plant operation no fears need be felt as to the possible effect of working in large-capacity fermenters.

(8) *Influence of Incorporating Additional Nitrogenous Nutrient to the Mashes, besides the Natural Nutrients of the Molasses itself.*—In our previous investigation on butanol and acetone production by fermentation, we had noticed a marked rise in yield of solvents from the use of sulphate of ammonia during mashing to the extent of 1 percent on weight of molasses used. Moreover, we also noticed a shortening in the duration of fermentation when this nutrient was added. These facts decided us to try the experiment in the present case, although our present yields were so high that they could hardly stand much improvement without reaching the theoretical limit.

For the experiment, seven sets of mashes were prepared. In six of these sets, ammonium sulphate was added in increasing quantities

from 0.5 gr. to 1.0 gr.; while set No. 7 was run as a check with no addition of this salt.

During the fermentation of the sets containing added sulphate of ammonia, the characteristic odor of hydrogen sulphide could be noticed in the incubator. This was not the case when the check set was fermenting nor had we noticed this phenomenon in our previous work. We concluded that the gas originated from the reducing action of the organism upon the sulphate of ammonia.

The generation of this obnoxious gas during fermentation would have been objectionable enough; but the fact that no beneficial effect could be observed from the use of the sulphate of ammonia, decided us against its further use.

Table No. 8 below presents the data obtained during the above experiment.

TABLE NO. 8

Mash Set No.	Ammonia Sulphate Added, Grams	Fermentation Time Hours	Residual Sugars %	Total Acidity Yield % Total Sugars	Generation of Hydrogen Sulphide
1	0 5	100	0.28	46.50	Yes
2	0 6	102	0.29	46.50	Yes
3	0.7	104	0.29	46.27	Yes
4	0.8	106	0.35	45 91	Yes
5	0 9	106	0.44	45.62	Yes
6	1.0	110	0.21	45.24	Yes
7	None	95	0.16	47.01	None

THE STANDARD MASH

The knowledge and experience gained through the work effected above, led us to the formulation of the "Standard Mash" for the production of Normal Butyric Acid from blackstrap. We had found that such a mash should possess the following characteristics:

a. A pH value of 6.8 to 7.0
b. Density of 10.6 deg. Brix
c. Total Sugar Concentration of 6.58 percent

and that its fermentation was to be carried at temperatures of from 30–32° C., without the necessity of inverting the sucrose or of incorporating any extraneous bacterial nutrient in addition to that already present in the blackstrap.

After some experiments, it was decided to make the Standard Mash as follows:

To one part molasses by weight, 7 parts water are added. Then to this diluted molasses, 0.135 part calcium carbonate is added. This mixture is autoclaved at 10 lbs. G. P. for 30 minutes, and after cool-

ing to the desired temperature, the mash is ready for inoculation. Determinations of pH in mashes thus prepared, gave values between 6.8 and 7.2; densities between 10.5 and 10.8° Brix and total sugar concentrations between 6.4 and 6.8 percent using average P. R. molasses.

To illustrate, suppose we wish to mash up 100 grams molasses: Add 700 grams water and 13.5 grams calcium carbonate; then sterilize and cool. Simpler mashing operations are not found anywhere in industrial fermentation work.

On Table No. 9 will be found the results of an "Over-All Efficiency Test" run with a series of ten mashes prepared according to directions given above for the "Standard Mash".

The analysis of the blackstrap was:

Degree Brix	85.60
Total Sugars (as Invert)	52.50
Nitrogen percent	0.80
pH value	5.5

The analysis of the resulting mash was:

Degree Brix	10.80
Total Sugars (as Invert)	6.50
Nitrogen percent	0.11
pH value	7.1

TABLE No. 9

Mash No.	Molasses Used Grams	Total Weight Mash Grams	Total Sugars Content Grams	Calcium Carbonate Added Grams	pH Value Initial	pH Value Final	Fermentation Time Hours	Residual Sugars %	Residual Sugars Grams	Grams Sugar Used	Sugars Used % Total Sugars	Total Acidity Yield Grams	Total Acidity Yield % Total Sugars	Total A idity Yield % Sugars Used	Total Acidity % Mash	Fermentation Efficiency % Total Sugars	Fermentation Efficiency % Total Sugars Used
1.....	100	800	52.0	13.5	7.1	6.8	96	0.20	1.60	50.40	96.92	24.20	46.53	48.01	3.025	95.15	98.17
2.....	100	800	52.0	13.5	7.1	6.8	95	0.19	1.52	50.48	97.07	24.22	46.57	47.97	3.028	95.23	98.09
3.....	100	800	52.0	13.5	7.1	6.7	94	0.20	1.60	50.40	96.92	24.18	46.50	47.97	3.023	95.09	98.09
4.....	100	800	52.0	13.5	7.1	6.8	96	0.18	1.44	50.56	97.23	24.30	46.73	48.06	3.038	95.56	98.28
5.....	100	800	52.0	13.5	7.1	6.8	96	0.16	1.28	50.72	97.53	24.55	47.21	48.40	3.069	96.54	98.97
6.....	100	800	52.0	13.5	7.1	6.7	96	0.17	1.36	50.64	97.38	24.37	46.86	48.12	3.046	95.82	98.40
7.....	100	800	52.0	13.5	7.1	6.8	94	0.18	1.44	50.56	97.23	24.28	46.69	48.02	3.035	95.48	98.20
8.....	100	800	52.0	13.5	7.1	6.7	95	0.16	1.28	50.72	97.53	24.40	46.92	48.10	3.050	95.95	98.30
9.....	100	800	52.0	13.5	7.1	6.8	96	0.17	1.36	50.64	97.38	24.40	46.92	48.10	3.050	95.95	98.52
10.....	100	800	52.0	13.5	7.1	6.8	95	0.18	1.44	50.56	97.23	24.36	46.84	48.18	3.045	95.78	98.52

According to the data on Table No. 9, the bacillus butyricus used in this research, when working on "Standard Mash" under optimum conditions of fermentation, possesses the power to:

a. Finish the fermentation in from 94 to 96 hours.
b. Use from 96 to 97.5 percent of the sugar content of the mash in this time.
c. Give a yield equivalent to:
  (1) 46 to 47 percent on T. Sugars.
  (2) 47 to 48.5 percent on Sugars Used.
  (3) 3.00 percent on Mash Weight.
d. Show a fermentation efficiency (based on theoretically possible yield) of 95 to 96.5 percent on total sugars, and 98 to 98.5 percent on sugars used.

Acid Extraction From the Fermented Mash

The method of separating the acid from the fermented mash was as follows:

The first step was to try the reaction of the fermented mash towards litmus paper. If the reaction was acid, then the mash was brought to neutralization by the addition of calcium hydroxide or carbonate.

The neutral, or slightly alkaline mash, was then evaporated down to one-fourth its original volume, preferably under vacuum. At this point the thickened mash was treated with enough sulphuric acid to liberate the fatty acid from its calcium salt, and the mixture was steam-distilled until all of the volatile fatty acid had passed over. In this way a dilute aquous solution of the acid originally present in the mash was obtained. Usually, the acid was recovered from the water solution in the form of its barium salt.

Products of Fermentation

The fermentation produced by the butyric acid bacillus strain discovered and isolated by the writer when operating in blackstrap molasses results in the production of:

1. N. Butyric Acid
2. Hydrogen gas
3. Carbon dioxide gas

the reaction taking place being as follows:

$$C_6H_{12}O_6 + \text{Ferment} = CH_3.CH_2.CH_2.COOH + {}_2H_2 + {}_2CO_2$$

Of these three products the normal butyric acid is, of course, the most valuable; but mention must be made here that the other two

gaseous products may be made to combine through the agency of high temperature and pressure and of some suitable catalyst to produce the purest form of methanol known to industry.

The normal butyric acid is produced in almost theoretical yield, and of a splendid degree of purity—99 percent or better, pure butyric acid. And this fact constitutes the outstanding merit of this process. It is only on very rare occasions that a fermentation product is obtained in such an extraordinary degree of purity.

The writer, after becoming awared of this most extraordinary fact, wished to have his work checked by two independent laboratories. The laboratory of the Bureau of Chemistry at Washington, D. C., and the private laboratory of Dr. Donald F. Othmer, professor of industrial chemistry at the Brooklyn Polytechnic, were selected. Samples of about 200 grams each of the barium salt of the acid were sent to these laboratories. The two reports received were identical, both laboratories reporting the material as *practically pure* butyrate of barium. Dr. Othner's report further specified that the acid extracted from the barium salt proved to be 99 percent or over, pure butyric acid. That the impurities (if present) could not be determined due to their being present in traces, and the sample of material being so small.

The splendid purity of the acid obtained by this fermentation is of paramount importance should the attempt be made to develop the process in commercial scale. One of the most expensive stages of the plant work, that of purification, would be eliminated from the start. The highest grade of butyric acid now in the market only guarantees from 98 to 99 percent purity; and this is only obtainable in their case, after much and laborious refractionation and purification; while here we have a natural product of practically C. P. quality.

Other Fermentation Tests

The work so far described above, strengthened the belief in the writer's mind that his discovery and process could possibly be applied in commercial magnitude. With this idea in mind, new tests were planned and executed that would result in increasing its range of usefulness, or lowering the cost of producing the acid.

Due to lack of space, the new experiments performed will not be given in detail; but the results obtained will be briefly commented.

To test the ability of the organism to work without absolute sterilization of the medium, a series of experiments were performed in which pressure cooking of the mashes was eliminated. Instead, the

mashes were prepared by heating to 100° C. at atmospheric pressure during five minutes. The results obtained were in all respects comparable to those produced when working with absolute sterilization under pressure. This encouraged us to go a step further in the same direction, using no heating at all of the mashes before inoculating with the bacillus. Results were again entirely satisfactory comparing favorably with those obtained previously with total and partial sterilization of the medium.

The economic importance of these later tests is self-evident; for, should it be possible to duplicate the experimental results in large scale, normal butyric acid of the highest purity could be produced at extremely low price, not more than a few cents per pound.

Through the courtesy of Mr. Herman Schreibler, consulting chemist of Lansing, Michigan, a sample of final beet molasses was made available to the writer for experimental purposes. The work performed on sugar cane final molasses was duplicated using this beet molasses as raw material; the same satisfactory results being obtained as to ease of fermentation, high yield and purity, of the finished product.

Also, and more recently; through the courtesy of the "Sucesión Serrallés" of Ponce, P. R., the bacillus was put to work on samples of Distillery Slops, for the conversion of the residual sugars contained in this waste material into butyric acid. It is highly significant, both from a purely scientific and industrial viewpoints, that although the Distillery Slops furnished by the "Sucesión Serrallés" contained, on the average, only about one percent of residual sugars; the organism succeeded in transforming them into the acid, almost quantitatively.

Uses of Butyric Acid
(*Present and Future*)

Due to its present high price (varying between 35 and 80 cents a pound, according to purity of material and quantities bought) Normal Butyric Acid finds most of its use in the Ester Industry. In this field, its application is divided mainly between the Flavor Industry and that of Perfumery and Cosmetics.

In these above mentioned industries we find butyric acid derivatives as Rhodinol; Propyl; Pheny-Ethyl; Octyl; Linalyl; Iso-butyl; Geranyl; Citronellyl; Cinnamyll; Butyl and Benzyl Butyrates. Also Capryl-Butyric Acid and Butyric Ketone (Butyrone). The prices of these derivatives very between $1.35 and $32 per pound.

Butyric acid derivatives in an impure form are beginning to ap-

pear in the Solvents Industry. "Amyl Ketol" is one of these products, consisting mainly of Butyrone. It is used as a solvent for both cellulose acetate and for nitrate of high nitrogen content; it also dissolves caoutchouc, ester gum, cumarone, linseed and castor oils, glyceryl phthalate and other resins. It is miscible with hydrocarbons. It possesses a rather sweet odor resembling pineapples, and is non-toxic at normal working concentrations.

The future uses of butyric acid in industry, will depend, in a great measure upon the price at which the commodity may be made available. As possible fields of application we may mention the (1) Leather Industry where it would serve as a softener, deliming agent; (2) Lacquer Industry, in the manufacture of plasticisers; (3) Varnish Industry, in the manufacture of Dryers; (4) Cellulose Esters; and (5) Pharmaceutical and Drugs.

The writer has also, of late, been using butyric acid in the preparation of a high grade alpha-cellulose pulp from cane bagasse and foliage. Results to date are entirely satisfactory.

Acknowledgments

The writer gratefully acknowledges his indebtedness to Dr. D. F. Othmer, professor of chemical engineering at Brooklyn Polytechnic Institute for his painstaking work in checking up the purity of the acid produced, and for the general interest he has taken throughout all the time that this investigation was taking place; to Dr. W. L. Owen, consulting bacteriologist of Baton Rouge, La., for his kind advice and early appreciation of the industrial possibilities of this discovery; to the Bureau of Chemistry of Washington, D. C., for the corroborative analysis on the barium salt of the acid produced; and finally to Mr. Herman Schreibler, consulting chemist of Lansing, Michigan; through whose kindness and interest the work on final beet molasses was made possible.

Summary

1. The ultimate aim of this research was to find an inexpensive biochemical method for the production of Normal Butyric Acid from Blackstrap.

2. A native organism was found and isolated in pure culture which proved admirably adapted to this purpose.

3. Difficulties experienced by previous investigators were obviated in the course of the above described work.

4. The process is simple, inexpensive, and merits further efforts towards its industrial application and commercial exploitation.

5. The main end product of fermentation (Normal Butyric Acid) is obtained in nearly theoretical yield, and of a splendid degree of purity: 99 percent or better.

6. It is the writer's intention to apply for patent rights protection for his discovery and process.

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INVESTIGATIONS ON THE ROOT OF *MANIHOT UTILISSIMA* POHL

(PROGRESS REPORT)

By H. E. Cruz Monclova Ch. E.
Agricultural Experiment Station, Río Piedras, P. R.

The work appearing now, is the incomplete result of experiments undertaken in cooperation by the Divisions of Agronomy and Industrial Chemistry, of the Agricultural Experiment Station, on the cassava plant. The complete and final results will appear as soon as they are available.

Methods of Analysis

1. Determination of Hydrocyanic Acid.

The principal aims of the determination of hydrocyanic acid in the cassava root were two:

First.—To compare the hydrocyanic acid content of the varieties of cassava roots under study.
Second.—To find out if possible the hydrocyanic acid content of the cassava roots at different stages of their growth.

After several methods of analysis were tried finally Liebig's Method was selected and adapted to the cassava, as the easier and more accurate.

One hundred grams of sample are weighed and placed in a two-liter flask; together with 1 of liter distilled water and 20 cc. of H_2SO_4 of known specific gravity are added and the flask is connected to a condenser. The distillate is collected in a 500 cc. volumetric flask, into which have been placed 25 cc. of dilute NaOH solution. At least 500 cc. of the distillate should be collected to ensure that all the HCN in the sample is distilled off. After the distillation is finished an aliquot part is taken and titrated with $\frac{N}{100}$ $AgNO_3$. The residue in the flask is saved for the determination of starch and of fiber.

Some trial distillations were made in order to establish the least possible volume of the distillate to be collected for titration. By taking two consecutive portions of distillate of 250 cc. each, and titrating an aliquot of 50 cc. of each separately, it was found that not all the HCN in the sample was collected in the first 250 cc. of dis-

tillate passing over, but that there was yet a perceptible amount in the next 250 cc. portion of distillate obtained as shown by the following results:

Variety of Cassava	1st. Portion	2nd. Portion
Carlos Checo	2.9cc.	0.8cc.
Negrona Grande	3.2cc.	0.6cc.
Manuel Pichardo	2.7cc.	0.4cc.

On using $\frac{N}{10}$ solution of $AgNO_3$ for the titration it was found that it did not afford the most accurate results obtainable under the circumstances. Comparative results are shown below for titrations made with $\frac{N}{10}$ and $\frac{N}{100}$ solutions of $AgNO_3$.

Cassava Variety	$\frac{N}{10}$ $AgNO_3$	$\frac{N}{100}$ $AgNO_3$
Tapicurú	0.40cc.	3.4cc.
M. Pichardo	0.20cc.	1.6cc.
Cristalina	0.20cc.	1.9cc
New Orleans	0.10cc.	1.0cc.
Carlos Checo	0.15cc.	1.5cc.

Determination of Starch and Fiber

The residue from the distillation of the HCN contains in the solution all the starch present in the sample. It has been transformed into dextrose by boiling with the acid. Besides, this residue contains also the fiber of the sample.

The residue is brought to volume of 1 liter at 20° C and filtered. The specific gravity of the filtrate is determined at 20° C by means of pycnometer. From the specific gravity of the liquid, the starch can be calculated by means of a curve made beforehand, for pure dextrose, and taking into consideration all the factors affecting the results. The fiber is obtained after filtering all the liquid; it is washed carefully and then transferred to a tared crucible, and dried at 100° C.

	Growing Period—4½ Months			Growing Period—12 Months			
	Moisture %	Fibre %	HCN %	Moisture %	Ash %	HCN %	Starch %
Mameya S. A.	62.45	1.6276	0.03375	69.23	0.6327	0.0081	18.01
Ceiba S. A.	.....	.....	.....	61.36	.6239	0.0108	23.99
Brazil No. 1	.....	.....	0.02430	68.21	.7894	0.0108	.....
Brazil No. 2	.....	.....	0.02295	63 04	1.0955	0.0162	21.22
Aipi Manteiga	.....	.....	0 02700	61.62	.8736	0.0108	25.50
Naparica	.....	.....	0.03510	60.16	1.0443	0.0189	24 96
Basiorao	.....	.....	0 02565	58.31	.7839	0.0108	27.19
Tapicurú	.....	.....	0.04320	64.62	.7631	0.0216	.....
Carlos Checo	64 29	2 0361	0.02497	.....	.....	0.0108	.....
Negrona Grande	77 26	1.6009	0.02700	66.30	.5553	0.0135	18.54
Manuel Pichardo	67.54	2 3273	0.02092	59.45	1.0369	0.0108	.....
Negrona Agria	70.13	1.8276	0.03240	.....	.....	.....	.....
Peralta	68.13	2.4551	0.01417	65.00	.5907	0.0162	20.15
Madretaso	68.46	2.4074	0.03645	.....	.....	.....	.....
Goyo Vega	61.77	2.0796	0.01755	.....	.....	.....	.....
Aipi Maugí	66 04	.....	0 03722	.....	.....	.....	.....
Señora, está en la Mesa	67 24	.....	0.01687	59.92	.9742	0.0081	24.07
Mata Gato	70 94	2.1762	0.02497	.....	.....	.....	.....
Pancha	78.92	2.1194	0.02700	.....	.....	.....	.....
Cartagena 2da	66.95	1.4205	0.02362	.....	.....	.....	.....
Dame Más	66.53	2.5220	0.02362	.....	.....	.....	.....
Mocana 677	65.01	2.1970	0.03307	.....	.....	.....	.....
Negrona Chiquita	73.41	1.6836	0.01350	60.30	.7637	0.0108	26.92
New Orleans	66.24	.....	0.01552	62.98	.8124	0.0054	.....
Cristalina	68.49	.....	0.02362	62.48	.9477	0.0108	.....
Puerto Plata	66.44	.....	0.2160	63.37	.9507	0.0081	23.00
Valencia	66.10	.....	.....	58.34	.9586	0.0135	27.64
Cartagena Santo Domingo	66.88	2.4058	0.02700	.....	.....	.....	.....
Miraclelo	77.45	1.8706	0.02700	.....	.....	.....	.....
Amarilla	.....	.....	.....	62.27	.5999	0.01485	20.68
X—No. 1	.....	.....	.....	55.70	.7751	0.0108	28.71
X—No. 2	.....	.....	.....	47.11	.7673	0.0081	29.42
Ceiba Villalba	.....	.....	.....	68.44	.5159	0.0108	18.25
Mata Burro	.....	.....	.....	69.65	.5866	0.0108	16.40
X—No. 6	.....	.....	.....	55.03	.8570	0.0081	28.71
Pana-Borinquen	.....	.....	.....	57.55	.8661	0.0135	25.50
Morada Palo Rojo No. 1	.....	.....	.....	65.39	.7980	0.0270	22.73
Pana	.....	.....	.....	59.08	.7828	0.0162	27.64
Forastera	.....	.....	.....	65.29	.5162	0.0081	19.43
Blanquilla	.....	.....	.....	57.38	.5531	0.0243	28.71
Pata Paloma	.....	.....	.....	63.85	.5989	0.0162	27.64
Coreana	.....	.....	.....	60.27	.7403	0.0108	26.46
Coreana Amarilla	.....	.....	.....	61.44	.8148	0.0108	26.46
Seda	.....	.....	.....	57.32	1.3876	0.0135	.....

Description of the Roots of Cassava Varieties

The ideal root of cassava for industrial purposes, either for starch or flour making must possess a number of characteristics all contributing to a cheaper or better final product. The cassava root must have the proper chemical composition-high starch and protein content, low humidity, low fibre content. The rind must be thin; its color must be white. The peel or skin must be thin, smooth, easily detached and of very light color. The inside of the root must be low in coloring matter and when pulped must be easily washed; when dried it must remain white, with no extraneous coloring; it must yield flour or starch of pure-white color.

In no one variety of cassava do we find together all these characteristics. But we believe that by selection and breeding there can

be obtained a type that will be much nearer the ideal than the presently known varieties of cassava.

The ratio of length of root to diameter must be small as a relatively short and thick root is easier to handle in the plant than a long and light root.

We are giving here some of the characteristics of some of the varieties studied, grouped according to color, type and thickness of the skin, underskin, rind and pulp.

I. Varieties with White under-skin.

1. Mameya, S. A.

The skin is thin and rough of dark brown color, underskin white. The rind is medium thick. The pulp is white.

2. Ceiba, San Antonio.

The skin is very thin and rather smooth; and of brown color. Underskin is white. The rind is thin. Pulp is yellowish-white.

3. Brazil No. 1.

The skin is thick and rough of dark brown color. Underskin is white the rind is medium thick. The pulp is white.

4. Brazil No. 2.

The skin is thick and rough and of dark brown color. Underskin is white. The rind is medium thick. The pulp is white.

5. Aipi Manteiga.

The skin is thin and rough of very dark brown color. Underskin white. The rind is thick. The pulp is white.

6. Tapicurú.

The skin is thick and of dark brown color; underskin is white. The rind is thick. The pulp is white.

7. Negrona Agria.

The skin is thick, rough and dark brown. Underskin is white. The rind is very thick. The pulp is white.

8. Cartagena 2da.

Skin is thick, very rough and dark brown. Underskin is white. The rind is thin. The pulp is white.

9. Cartagena Sto. Domingo.

Skin is thin, smooth and of straw color. Underskin white. The rind is thin. Pulp is white.

II. Varieties with light-red Underskin.

10. Manuel Pichardo.

The skin is thin and smooth of light straw color. Underskin light-red color. The rind is thick. Pulp is white.

11. Peralta.

The skin is thin and smooth, light straw color. Underskin light-red color. The rind is thick. Pulp is white.

12. Machetazo.

The skin is thin, medium rough and brown. Underskin is light-red color. The rind is thin. Pulp is yellowish.

13. Señora está en la Mesa.

The skin is thick, rough and dark brown. Underskin is light-red color. The rind is thin. Pulp is white.

14. Dame Más.

The skin is thick, rough and dark brown. Underskin light-red color. The rind is thick. Pulp is white.

15. Mocana 677.

The skin is thick, smooth and dark brown. Underskin is light-red color. The rind is thin. The pulp is white.

16. Negrona Chiquita.

The skin is thick, medium smooth and dark brown. Underskin is light-red color. The rind is thick. The pulp is white.

17. New Orleans.

The skin is very thin, smooth, and of light straw color. Underskin is light-red color. The rind is thin. The pulp is white.

18. Cristalina.

The skin is very thin, very smooth and of light straw color. The underskin is light-red color. The rind is thin. The pulp is white.

19. Puerto Plata.

The skin is thin, smooth and of light straw color. The underskin is light-red color. The rind is of medium thickness. The pulp is white.

III. Varieties having Red Underskin.

20. Miracielo.

The skin is thick, rough and of dark brown color. The underskin is red in color. The rind is thin. The pulp is white.

21. Valencia.

The skin is thick, rough and of very dark brown color; underskin red in color. The rind is thick. The pulp is white.

22. Aipi Mangi.

The skin is thick, rough and of dark brown color. The underskin is red in color. The rind is thin. The pulp is white.

23. Goyo Vega.

The skin is thick, rough and dark brown color. The underskin is red in color. The rind is thick. The pulp is white.

24. Negrona Grande.

The skin is thick, rough and dark brown color. The underskin is red in color. The rind is thick. The pulp is white.

25. Carlos Checo.

The skin is thick, rough and dark brown in color. Underskin is red in color. The rind is thick. The pulp is white.

The results given in this preliminary paper are not final nor complete. Much more work has to be done yet to have the complete chemical and industrial data on the root of the cassava. From the data gathered, however incomplete, certain conclusions can be derived, especially those referring to moisture content and to hydrocyanic acid content. It appears that the water content of the root decreases with its age; obeying the same variation as the hydrocyanic acid content. These variations in general are not of the same degree for different varieties.

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THE RELATION OF BUFFER CAPACITY AND ORGANIC MATTER TO THE SOLUBILITY OF THE NUTRIENT ELEMENTS IN TOA SILT LOAM

By ARNALDO VÉLEZ FRANCESCHI *

ABSTRACT

The reaction of arable soils varies widely. Since each soil is affected and reacts differently from all others to applications of fertilizers and soil amendments, it is the problem of the soil scientist to make recommendations regarding the value of different soil amendments and fertilizers on the basis of individual soil type. It has been the purpose of this work to study the effect of buffer capacity and organic matter on the solubility of the mineral nutrient elements in Toa silt loam. This is an alluvial soil from the northern coast of Puerto Rico. Sugar cane is the universal crop grown on it. This soil type is very fertile. Since the buffer capacity, resistance to change in reaction, or pH, of Toa silt loam has not been extensively studied it was desired to study it more thoroughly.

The six samples of Toa silt loam received the same treatments throughout the experimental procedure. Each sample was treated with successive increments of a saturated solution of calcium hydroxide or tenth normal sulfuric acid, each addition being equivalent to one, two and three tons of calcium carbonate or sulfuric acid equivalents of calcium carbonate per acre, respectively.

It was found that each sample of Toa silt loam behaved differently in the solubility of its mineral nutrient elements. This behavior was largely dependent on the amount of organic matter present in the sample. Some of the nutrient elements like potassium and manganese were found to be more soluble at higher pH values whenever the sample in question was high in organic carbon content. In samples in which the organic carbon content was not as high the solubility of these two nutrient elements was found to increase only at lower pH values. Liming increased the solubility of calcium but did not decrease the solubility of phosphorus although it always decreased the solubility of potassium, manganese, and iron. Additions of acid always increased the solubility of calcium, magnesium, manganese, iron, and potassium. It was found that in samples high in

* Agricultural Extension Agent, Isabela, P. R. Thesis submitted to the faculty of the Louisiana State University and Agricultural and Mechanical College as a partial fulfillment of the requirements for the degree of Master of Science.

organic carbon content the buffer capacity, resistance to change in reaction, was greatest near neutrality. In the samples with a lower per cent of organic carbon the buffer capacity was found to be greatest at the extreme ranges of acidity and alkalinity. This was represented by pH curves plotted against the amount of acid or base used.

This work bears out the fact that the ability of a soil to resist a change in reaction, buffer capacity, is an individual characteristic for each soil. Without a knowledge of the buffer characteristics of the soil, no definite recommendations can be set forth regarding the effect of a particular fertilizer or soil amendment on the soil. This is of great economic importance to the crop grower since additions of fertilizer materials, both acidic and basic, or soil amendments to the soil is a very common practice in the agricultural field. The effect of additions of acidic or basic materials to the soil will not produce the same results in any two soils. The profits to be derived therefrom will be proportional to the power of the soil in question to yield soluble mineral nutrient elements to be used by the plant grown.

Introduction

The reaction of arable soils varies between pH 4.0 and 9.0 and depends upon the relative amounts of hydrogen, calcium, and sodium adsorbed by the humus and mineral or clay colloids of the soil. Since the humic or clay acids which form weak combinations with adsorbed cations of hydrogen, sodium, or calcium, are unlike normal acids and have no very definitely fixed capacity for combining with bases, they will exhibit the strong buffer capacities characteristic of weak acids or the calcium or sodium salts of weak acids. The strong buffering effect of the soil acids or salts of the soil acids is due to the fact that only a very small fraction of the acid or salt is dissociated at any one time to give hydrogen, calcium or sodium ions, but increasing amounts dissociate and are neutralized when increasing amounts of base or acid are added. The complexity of these colloidal acids or their salts in the soil makes necessary the use of indirect methods for the study of the acidic or basic properties of a soil. These properties are most conveniently and practically measured by curves which show the pH values plotted against the amounts of standard acid or base which has been added to the soil in question.

Different soils contain widely varying amounts and proportions of the humus and clay colloids and the interaction of the humus and clay in different soils varies. There are also changes in the saturation capacities of the soil colloids with changes in the soil re-

action or pH values. Thus the determination of the quantities of bases or acids that must be added to a soil to effect a unit change in reaction is an individual problem for each soil.

Associated with the change in reaction of a soil is the change in the solubilities of the mineral elements in the soil. The changes in the solubilities of the nutrient elements are of especial practical importance. The current practices in the cultivation of crops involve the addition to the soil of both acidic and basic substances as fertilizers or soil amendments. One of the most common basic materials used is lime in some of its forms while the most common acidic materials are ammonium sulfate, sulfur, ammonium chloride, and sulfuric acid.

The study of the effect of the change in reaction of a soil largely resolves itself into a determination of the unit changes in reaction, or pH value, per unit addition of some commonly used basic or acidic substances and the effects of these substances on the solubilities of the nutrient elements. The effects of such additions will be specific for a given soil under a definite set of conditions. Since the buffer capacity, or the resistance to change in reaction, for Toa silt loam has not been extensively studied, it is the purpose of this work to study the effects of additions of calcium hydroxide and sulfuric acid on the changes in reaction of the soil and the solubilities of the mineral nutrient elements in six different samples of Toa silt loam which vary widely in their humus or organic matter content.

Review of Literature

Comprehensive studies made by Pierre and others (17, 18, 19) show that in most investigations on buffer action of soils the results have been recorded as titration curves representing the amount of acid and base used against the pH obtained. Charlton (17) suggested a method giving fixed numerical values to the buffer capacity of soils by using simple formulae. He states that total buffer capacity toward acid is the amount in milliliters of normal sulfuric acid used to bring 100 grams of soil to pH 4.5. Similarly, he states that the total buffer capacity toward base is the amount in milliliters of normal barium hydroxide used to bring 100 grams of soil to pH 9.5. However, this method is open to question due to the fact that original pH values of soils vary, preventing the indication of the relative power of different soils to buffer given amounts of acid or base. Therefore, Charlton indicated two other expressions which he named "buffer action per 1.0 pH toward acid and toward base". He defined the former

as the total buffer capacity divided by X-4.5, where X is the original pH of the soil. The buffer capacity toward base is the total buffer capacity divided by 9.5-X. He called this "buffer capacity per 1.0 pH" the "specific buffer capacity".

Pierre and Worley (**18**) found that liming soils in accordance with the amount of exchangeable hydrogen brought the soils to pH 6.5. They also found the average liming factor at pH 6.5 to be 1.43 and at pH 6.0 the average liming factor was 1.52 and so concluded that the exchangeable hydrogen method, unlike the buffer method, gave no indication of the lime needed to bring the soils to pH values other than 6.5.

Runk (**23**) making a lime requirement survey for Delaware soils found that the lime requirements obtained in the laboratory methods could not be applied in the field without harming the crops. It was found that in some instances lower yields were evident and in others yellow color and stunted leaf growth were observed. However, when smaller applications than those indicated by the laboratory tests were applied to these soils very satisfactory results were obtained. Runk concludes that since the differences in buffer capacities of soils are so varied, "it would seem necessary to know the buffer capacity before applying materials which affect soil reaction".

Slipher (**26**) found that while applications of lime were arithmetic, the crop responses were geometric. He observed a similar result in the change of soil reaction, pH, by lime. The first addition of lime increases the pH more than does each successive addition applied in multiple additions. The efficiency of lime on a loamy soil was less than on a sandy soil. This he ascribed to the stronger buffer capacity of the loamy soil. Slipher concludes that it appears advisable to use lower rates of liming than have been commonly advised in the past.

Turner (**28**, **29**, **30**) shows that the buffer capacity of humus or its capacity for holding cations per unit weight is about six to seven times that of clay and that the exchange capacity of humus is not greatly affected by the presence of clay. When the ratio of clay to organic matter exceeds 20, the organic matter may be present almost entirely as films on the inorganic or clay particles. The saturation deficits of the good soils can be taken as a guide to the amount of hydrolysis which can take place in them, after they attain equilibrium with calcium carbonate. In the same way, their degree of unsaturation is a measure of the proportion of acidoid material unneutralized under these conditions. Turner shows that the quantities of lime requirements based on the extent of unsaturation of the good soils

are equal to the requirements of lime needed to bring the soil into equilibrium with calcium carbonate. The amounts of lime required vary with the degree of unsaturation of the soil. There exists a tendency of the soil reaction to decrease as the soil increases its unsaturation capacity. Magistad, Joffe, and McLean (**29**) have shown that the solubility of iron and aluminum in soils is dependent on soil reaction as well as upon the nature and amount of the anions present. Iron and aluminum do not always come into solution when acid soils are leached with neutral salt solution; the reasons determining their presence or absence are not fully understood. Turner found no replaceable iron, in some instances a trace was visible, in many soils having low pH values but higher saturation deficits. Iron was not found in the filtrates unless the saturation deficit was as high as 10 to 12 milligram equivalents per 100 grams of soil.

McGeorge (**10**) found a close relationship between the exchange capacity and the carbon content of soil. From his work it appears that humus overpowers the effect of clay.

Wilbur, Leighty and Shorey (**32**) observed that the carbon-nitrogen ratio varies considerably in soils. They found that the carbon-nitrogen ratio had a relation to the amount of organic matter present as food for micro-organisms. A ratio of 20:1 was found to indicate a fair amount of decomposable organic matter while a 10:1 ratio, or less, shows an advanced state of degradation of organic matter. They concluded that it was useless to attempt multiplying the total nitrogen by the factor 20 to calculate the organic matter.

Hissink (**6**) noted that in humus soils the buffer capacity increased near the neutral region. He found also that the buffer capacity (lime required to change pH by 0.1) per unit humus content was independent of the actual humus content of soils examined.

Kuchinskii (**7**) found that the buffer capacity of soils was almost zero after the clay particles had been separated from the soil. The humus portion of the soil exhibits a high buffer capacity. The greater the buffer capacity the greater the base exchange capacity. This investigator found some relation between hydrolytic acidity and buffer capacity.

McGeorge (**11**) showed that the exchange capacity of the organic portion increases as the organic matter decomposes. This is due to the fact that the lignin portion of the soil organic matter is most resistant and has the highest exchange capacity.

Morgan (12) observed that soil texture and organic matter content bear a close relationship to the calcium carbonate adsorption

factor. It was noted that clay and organic matter each increased the value of the factor.

Myers and Gilligan (**13**) concluded that the acidity of a soil, pH, does not indicate the extent of saturation of the colloidal acids and bases and that the pH values of different soil types do not show the relative buffer capacities of the soils in question. They studied the mechanism of buffer action and explain it as follows: "When acid is added to a soil it reacts with the salts of colloidal acids forming colloidal acids and crystalloidal salts. The resultant reaction of the system is only lightly altered in consequence of the weakness of the colloidal acids. When acid is added in excess of that required to react with the cations of the colloidal salts, the crystalloidal salts formed, having an ion in common with the added acid, tend to repress the ionization of the latter. The net result is a small change in the H-ion concentration of the soil dispersion."

Nemec and Gracanin (**14**) observed that the larger adsorption of potash and phosphoric acid after additions of calcium carbonate was seen only in acid soils (deficient in calcium oxide), but soils containing a fair supply of calcium oxide showed a decrease in the reabsorption of potash and phosphoric acid. These investigators noted that soils containing small amounts of available phosphoric acid respond markedly to calcium carbonate, insofar as the reabsorption of phosphoric acid was concerned, but that in soils with large amounts of available phosphoric acid the calcium carbonate inhibited the reabsorption of the phosphoric acid. The same results were found for potash, but were not as regular.

Pierre, Pohlman, and McIlvaine (**19**) found organic soils to contain low quantities of aluminum even though very acid and that large amounts of organic matter check the harmful effects of soluble aluminum on sensitive crops. These authors showed that there may be less soluble aluminum on acid soils of high percentage base saturation than in soils of low percentage base saturation. The authors showed that the amount of soluble aluminum in different soils of same pH values varied greatly.

Pryanishnikov (**20**) working with a chernozem soil and three types of podzols adjusted to different reactions (from pH 4.5–8.0) grew plants on them. He found that each soil behaved differently. Mustard plants failed to grow at a pH of 4.5 on a sandy soil but grew at the same reaction on a sandy loam. He concluded that soils with high buffer capacity can support plants even at low pH values. This investigator observed that water extracts from acid soils contained large quantities of calcium, which seemingly arrested the del-

eterious effects of the acids. It was noted that the intake by the plants of this calcium in the acid cultures was obstructed when ammonia was used as a source of nitrogen. He showed that a similar increase of magnesium and potassium in the nutrient solution had an effect much like that of calcium, but not as high. The mobilization of phosphorus and its utilization on limed podzol could be ascribed to the calcium.

This effect of calcium has been noted in the United States, particularly when small applications of limestone have been applied to acid soils with or before superphosphate.

Ganssen (**4**) observed that humus increases the solubility of phosphates in the soil by decreasing the proportion of active alumina.

The review of literature shows that a study of the buffer capacity of soils resolves itself into an individual problem for each soil. No general recommendation as to the amounts of lime or acidic material which must be applied to a given soil to effect a definite change in the reaction of the soil can be predicted accurately except by tests on the soil. Furthermore, the change in the reaction of the soil caused by the use of an amendment or of a basic or acidic fertilizer is of primary practical importance in its effect on the solubility of the nutrient elements in the soil. The solubility of the nutrients will vary according to the buffer capacity and other associated characteristics of the soil. Since the buffer capacity of Toa silt loam has not been extensively studied, it was desirable to investigate its buffer capacity as related to its organic content and changes in the solubilities of the nutrient elements as affected by additions of lime and sulfuric acid.

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of Soils, Insular Experiment Station, Río Piedras, Puerto Rico, for sending the soil samples and for other useful information.

Experimental Methods

The soil used in this work was six different samples of Toa silt loam. The Toa soils occur very extensively on nearly all the streams of the north coast of Puerto Rico (**21**). "The Toa soils have developed from material washed from the shale and limestone hills which has been deposited in the stream-flood plains. They are grayish brown to brown in color, silty in texture, nearly uniform in both color and texture to a depth of several feet. All layers are nearly neutral to slightly alkaline."

"Physically and chemically these soils are among the best sugarcane soils in Puerto Rico. They have a nearly level surface relief, good drainage and are almost ideal for maximum agricultural utilization. These soils permit the cultivation of all the land and the use of any kind of modern machinery. The surface soil is loose and porous and is easily cultivated. Water can penetrate it rapidly and there is enough silt and clay in the subsoil to retain large quantities of moisture. These soils have considerable organic matter and plant food, yet fertilizer and lime bring very good returns. This soil will support a wide diversification of profitable crops, but sugarcane seems to be the most profitable as nearly 100 per cent of the land is planted to cane. Yields vary from 30 to 50 tons but field observation indicates that in many places these yields can be doubled with irrigation, proper handling, good fertilizer rates, and, in some cases, liming."

The six samples of Toa silt loam studied were taken at a depth of six inches in the following location:

I. Central Constancia, 2.5 kilometers north of Toa Baja. Taken at six representative places on March 11, 1931.

II. Central Canóvanas, 0.5 kilometers east of main office. Taken at six representative places on March 5, 1931.

III. Finca Nevares, 1.8 kilometers on Toa Baja road. Taken at six representative places on March 11, 1931.

IV. Finca San Antonio, Bayamón. Taken at six representative places on March 11, 1931.

V. Kilometers 26.6, Río Grande–Mameyes road (left), side-road El Verde, finca Tamarindo. Taken at three representative places on March 5, 1931.

VI. Finca El Naranjal, 1 kilometer north of Carolina. Taken at three representative places on March 5, 1931.

All samples were taken by J. A. Bonnet, Chief of the Division of Soils, Insular Experiment Station, Río Piedras, Puerto Rico, and were air dried and passed through a 2 mm. sieve.*

The buffer effect towards lime was determined by the addition of successive increments of a saturated solution of calcium hydroxide to 50-gram portions of soil at rates beginning with the equivalent of one ton calcium carbonate per acre, 0.05 per cent, and increasing to three tons per acre, 0.15 per cent.

After adding the saturated solution of calcium hydroxide, the samples were stirred for five minutes at thirty-minute intervals for half a day. Percival (16) found that by aerating samples after additions of lime had been made, equilibrium was attained during 20 to 24 hours. Since the laboratory equipment did not permit this procedure, intermittent stirring was resorted to and in this way the carbon dioxide was driven off and complete mixing of the soil and lime was obtained. After the addition of the lime increments all the solutions were made up to 73.5 milliliters with water, which was the volume of the largest addition of calcium hydroxide. All samples were evaporated to air-dry condition at room temperature.

Similar procedure was followed in determining the buffer effect towards acid. Tenth normal sulfuric acid was added in increments which were the equivalents of calcium carbonate at the rates of 1, 2, and 3 tons per acre.

The treatments given to each sample of soil were as follows:

1. 50 grams soil plus 30 ml. tenth normal sulfuric acid, equivalent to 2.94 tons sulfuric acid per acre.
2. 50 grams soil plus 20 ml. tenth normal sulfuric acid, equivalent to 1.96 tons sulfuric acid per acre.
3. 50 grams soil plus 10 ml. tenth normal sulfuric acid, equivalent to 0.98 tons sulfuric acid per acre.
4. No treatment. Check.
5. 50 grams soil plus 24.5 ml. limewater, equivalent to 1 ton calcium carbonate per acre.
6. 50 grams soil plus 49 ml. limewater, equivalent to 2 tons calcium carbonate per acre.
7. 50 grams soil plus 73.5 ml. limewater, equivalent to 3 tons calcium carbonate per acre.

The 50-gram portions of air-dried soil were transferred to 500 milliliter Erienmeyer flasks with 250 milliliters of freshly distilled water. The samples were shaken for 5 minutes and filtered through

* The main purpose in obtaining those samples was to use them in nitrification studies published in Bonnet, J. A. 1935. Nitrification studies with soil types of northern Puerto Rico. Jour. Agric. Univ. P. R. 19(2): 73–103.

neutral quantitative filter paper and the following determinations were made on the filtrate:

1. pH.
2. Soluble Ca and Mg.
3. Soluble P.
4. Soluble K.
5. Soluble Fe and Mn.

Total carbon was determined by the dry combustion method of Winters and Smith (**34**),

Total or protein nitrogen was determined by the Gunning-Hibbard method as modified by Bal (**3**).

The solubility of phosphorus was estimated by the method of Parker (**15**).

Potassium was determined gravimetrically by the sodium cobaltinitrite method as outlined by Van Rysselberge (**31**).

Calcium and magnesium were estimated by the turbidity methods as given by Schreiner and Failyer (**25**).

Iron was measured colorimetrically as proposed in Bulletin 31, U. S. Dept. Agr. Bureau of Soils (**25**).

Manganese was determined by the periodate method of Greathouse as described by Yoe (**35**).

Reaction or pH was determined colorimetrically, using standard indicators and Hellige standard color discs.

Experimental Results and Discussion

From Table 1 can be seen the changes in the solubilities of the different elements according to the corresponding changes in reaction or pH. Soil I is high in soluble calcium, the check having 384 p.p.m. at a pH of 6.7. In general, Soil I has a high buffer capacity, the lowest pH value attained being 5.0 and the highest being 8.0. The solubility of calcium was doubled from the first two additions of acid. With the third increment, the equivalent of three tons of sulfuric acid per acre, the solubility of calcium increased almost threefold over that in the check. In the alkaline range, the solubility was twice that of the check but the second and third increments, two and three ton equivalents of calcium carbonate, gave the same solubility of calcium. The solubility of magnesium decreased as the pH decreased but increased slightly over that of the check on the alkaline side with the addition of the third increment, the equivalent of three tons of calcium carbonate per acre. Soluble iron was found only as a trace on the acid side. Phosphorus became more soluble

as the acidity increased. On the alkaline side its solubility increased as the pH increased but never attained the magnitude of solubility shown on the acid side. Potash was very soluble at low pH values, becoming less soluble as alkalinity increased.

TABLE 1

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, I

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p p. m.	P p. p. m.	K p. p. m.	pH
1	1040	160	46	trace	3.6	110	5.0
2	680	173	18	trace	3.3	90	6.1
3	671	173	no test	trace	1.8	43	6.2
4	384	208	no test	no test	2.5	15	6.7
5	454	104	no test	no test	1.2	13	7.2
6	769	208	no test	no test	1.7	18	7.8
7	769	346	no test	no test	2.5	18.1	8.0

* On the basis of dry soil.

In general, the solubility of the nutrient elements in this soil decreased with additions of lime. This soil was the second highest in organic carbon content, 2.25 per cent, (Table 7). It ranked second also in total nitrogen, 0.235 per cent. It can be seen from a comparison of the curves showing the buffer capacities of the several samples and from the organic contents shown in Table 7, that an increase in the soil organic matter increases the buffer capacity near neutrality, which is reflected in a flatter curve.

TABLE 2

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, II

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p. p. m.	P p. p. m.	K p. p. m.	pH
1	453	231	59	trace	1.7	40	3.9
2	250	181	48	trace	2.5	30	4.0
3	177	137	25	no test	3.1	30	4.6
4	40	52	9.3	no test	2.8	10	6.0
5	67	119	8	no test	3.6	20	6.9
6	100	133	14	no test	3.1	10	7.2
7	100	166	trace	no test	3.1	none	7.2

* On the basis of dry soil.

Table 2 shows the behavior of Soil II. The check was low in soluble calcium, 40 p.p.m. As in Soil I, lime increased in solubility as the pH became lower. Additions of lime increased the solubility of calcium over that in the check slightly, but on the acid side the solubility of lime increased tenfold with the addition of the three tons equivalent of sulfuric acid per acre. The changes in the solubility of magnesium were like those of calcium except for smaller

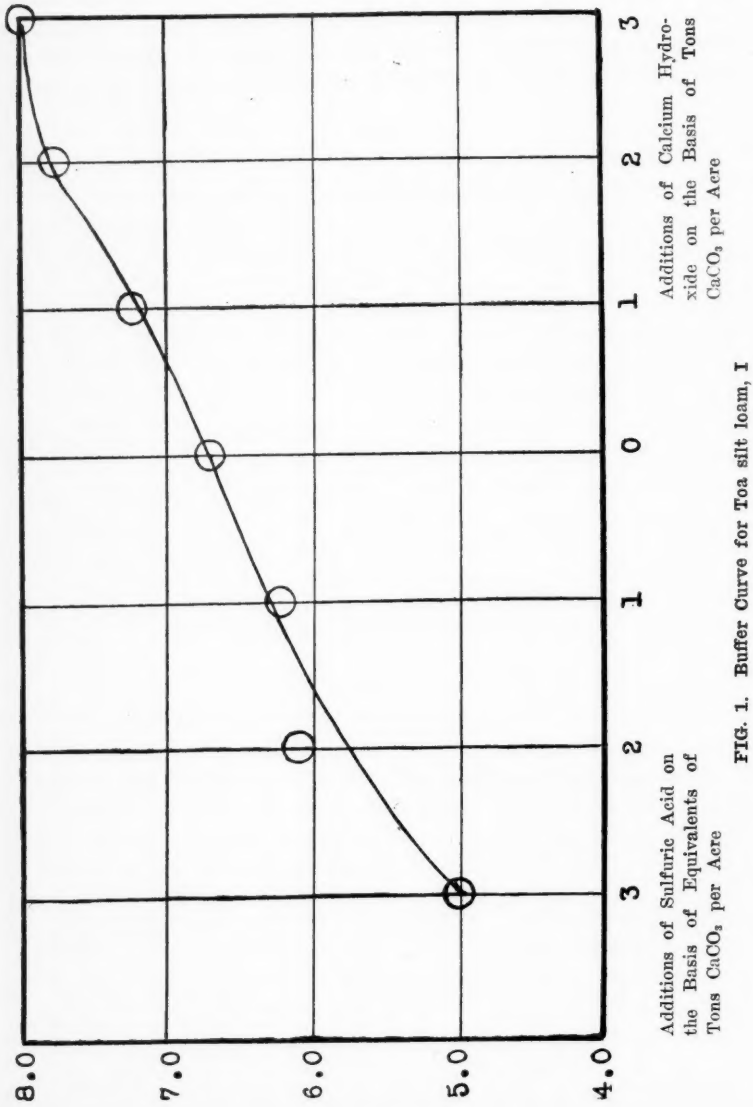

FIG. 1. Buffer Curve for Toa silt loam, I

differences. The soil seemed to maintain about a 1:1 ratio of Ca:Mg at neutrality. Soil II, like Soil I, was high in soluble manganese. This nutrient element was more soluble on the acid range, increasing its solubility as the pH decreased. Manganese was present in the check, (pH 6.0), and in the treatments with the first two increments of lime. There was no appreciable increase in the pH from the addition of the third increment of lime, yet, only a trace of manganese was present as compared to 14 p.p.m. present in the two ton treatment or second increment. There was only a trace of iron at a pH of 3.9 and 4.0. These correspond to the additions of three and two tons of sulfuric acid per acre, respectively. No test for soluble iron was obtained at other pH values in Soil II. The solubility of phosphorus increased with the one ton per acre application of sulfuric acid but in the successive applications the solubility decreased and was less than that of the check. With the addition of one ton of calcium carbonate the solubility of phosphorus was the highest of all, 3.6 p.p.m., this being 0.8 p.p.m. over the check. However, the two and three ton applications of lime decreased the solubility from 3.6 p.p.m. to 3.1 p.p.m. for each application. (It should be mentioned here that the greater solubility of phosphorus on the alkaline range may have been due to the colloidal state of the filtrates since it was impossible to get clear filtrates in the alkaline range.) Potash increased in solubility as the pH decreased. The solubility on the alkaline side was twice that of the check from the one ton treatment of calcium carbonate but decreased thereafter.

Soil II was the lowest in organic carbon content, 1.19 per cent, (Table 7) and it also ranked last in total nitrogen, 0.126 per cent. It is very obvious from the buffer curves of Soils I and II and from Table 7 that Soil I which has a relatively high organic content is highly buffered near neutrality, while Soil II which has a low organic content is lightly buffered near neutrality but is highly buffered at the extreme ranges.

TABLE 3

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, III

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p. p. m.	P p. p. m.	K p. p. m.	pH
1	625	92	6.3	no test	1.4	60	4.0
2	525	87	7.8	no test	1.2	80	4.3
3	500	106	trace	no test	1.1	50	4.7
4	145	55	no test	no test	1.5	10	6.0
5	165	81	no test	no test	1.4	10	6.5
6	190	87	no test	no test	1.6	10	6.8
7	275	100	no test	no test	1.6	30	6.9

* On the basis of dry soil.

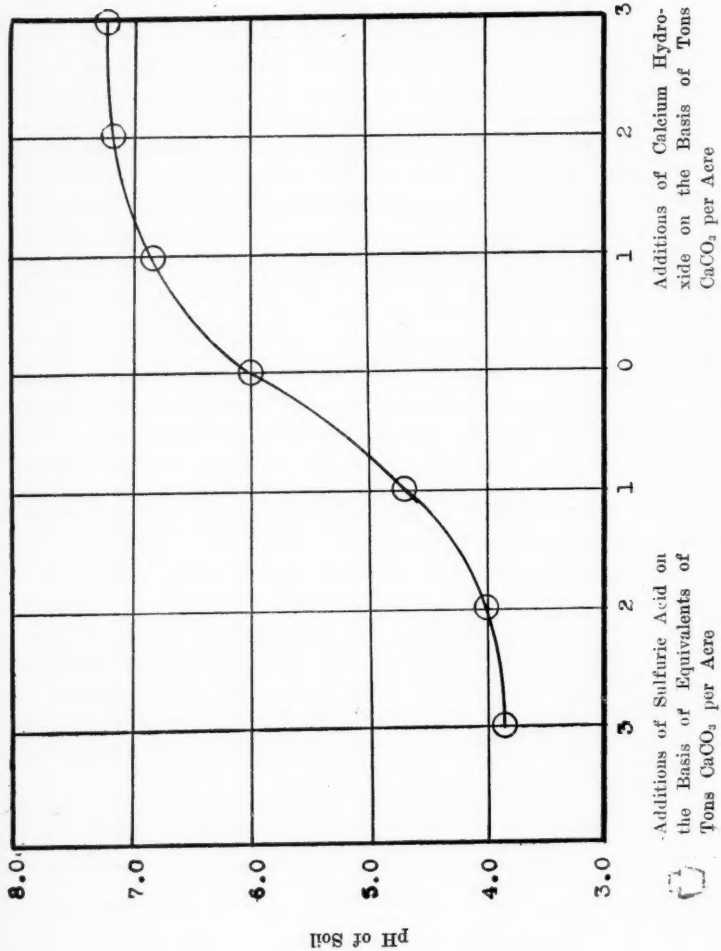

FIG. 2. Buffer Curve for Toa silt loam, II

Table 3 shows that Soil III was high in calcium which became more soluble as the acidity increased. The additions of lime also increased the solubility of calcium over that of the check, but to a lesser degree than the increase from the additions of sulfuric acid. Magnesium showed relatively less changes in solubility from additions of either acid or base. There was not much soluble manganese in this soil. At pH 4.3, after the addition of two tons of sulfuric acid per acre, there was 7.8 p.p.m. of soluble manganese, present. The solubility of manganese was too low to be measured above a pH of 4.7 in Soil III. No test for soluble iron was obtained for this soil. Phosphorus was slightly more soluble on the alkaline side than on the acid side. As in Soil II, this may be ascribed to the colloidal state of the alkaline filtrates, it being impossible to get clear filtrates. Potash was six times more soluble at a pH of 4.0, three tons of sulfuric acid per acre, than the check. The highest solubility was attained at pH 4.3, two tons of sulfuric acid per acre. The three ton addition of lime increased the solubility of potash over that of the check.

Soil III, like Soil II, is low in organic matter, (Table 7), and shows the same peculiar property in buffering the effects of acid and base; that is, the soil is much more highly buffered at the extreme ranges than near neutrality.

TABLE 4

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, IV

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p. p. m.	P p. p. m.	K p. p. m.	pH
1	555	104	44	no test....	0.8	50	4.0
2	417	173	26	no test....	0.8	10	4.3
3	345	148	9	no test....	1.1	10	5.0
4	83	139	no test....	no test....	1.9	10	6.1
5	167	173	no test....	no test....	1.7	none.....	6.9
6	167	104	no test....	no test....	1.7	none.....	7.2
7	227	130	no test....	no test....	2.0	none.....	7.3

* On the basis of dry soil.

Table 4 shows the data for Soil IV. This soil was low in lime content but the solubility of lime increased sixfold at the lowest pH, 4.0, (three tons sulfuric acid per acre), over that of the check. Lime additions caused a slight increase in the solubility of calcium. The solubility of magnesium was slightly altered on the acid side but it decreased with the second and third applications of lime. Soil IV was as high as Soils I and II in its manganese content but in this particular soil manganese was soluble up to a pH of 5.0 only. No test for soluble iron was obtained for this soil. Phosphorus was ap-

FIG. 2. Buffer Curve for Toa silt loam, II

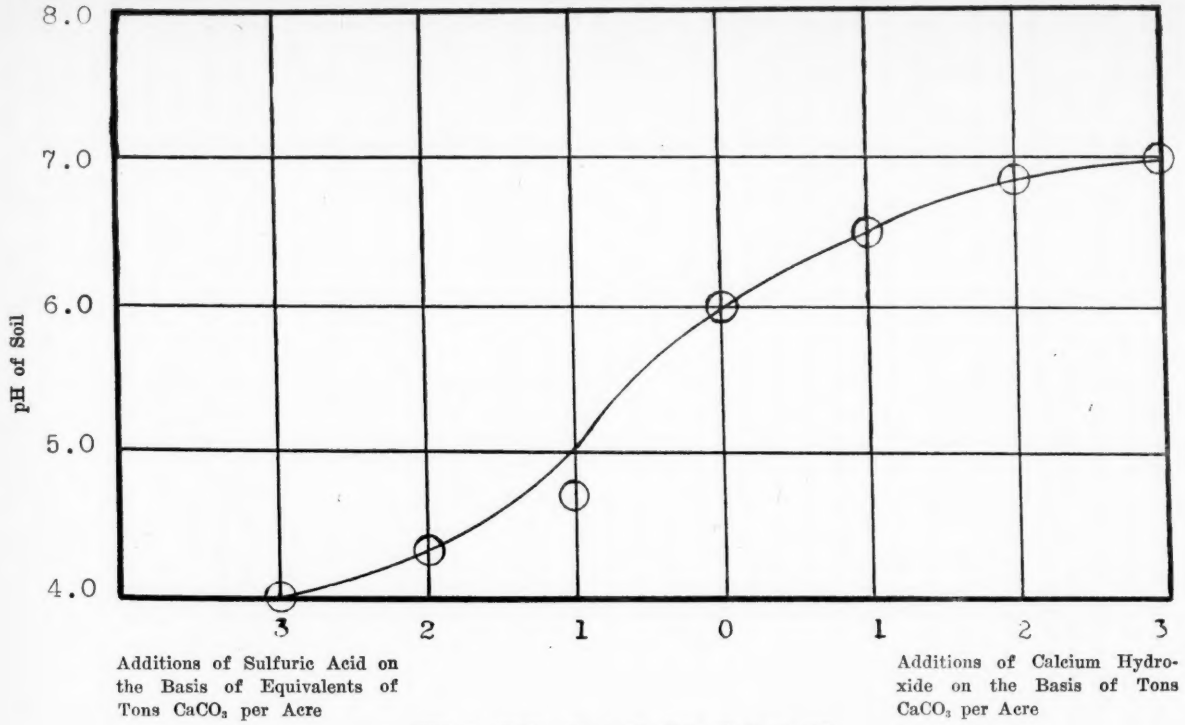

FIG. 3. Buffer Curve for Toa silt loam, III

parently less soluble on the acid side. The turbidity of the filtrates, as explained before, may account for this. This soil was low in potash content 10 p.p.m. The first two additions of sulfuric acid, (one and two tons per acre, respectively), failed to affect the solubility of potash but the third increment increased the solubility five times over that of the check. On the alkaline range the solubility was too little to be measurable.

It can be noted that Soil IV, like Soil II and III, was low in organic matter content. Like Soils II and III, this soil shows a low buffer capacity near neutrality and a high resistance to change in reaction at the extreme ranges. The solubilities of phosphorus and potassium are very low in this soil. This may be due to the high buffering power shown at the extreme ranges.

TABLE 5

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, V

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p. p. m.	P p. p. m.	K p. p. m.	pH
1.....	555	187	trace.....	no test....	1.6	7	4.2
2.....	450	148	trace.....	no test....	1.2	10	4.9
3.....	450	148	no test....	no test....	1.3	20	5.4
4.....	710	173	no test....	no test....	1.3	7	6.5
5.....	710	187	no test....	no test....	1.6	10	6.5
6.....	500	104	no test....	no test....	1.9	7	7.2
7.....	355	159	no test....	no test....	1.6	10	7.1

* On the basis of dry soil.

The differences in solubilities of the nutrient elements at varying pH values for Soil V can be seen in Table 5. This soil is the highest in soluble lime content, 710 p.p.m. The additions of acid did not increase the solubility of calcium. The same was evident with the additions of lime but to a larger extent. The solubility of magnesium did not vary appreciably on either range. Only a trace of manganese was seen at pH values of 4.2, (three tons of sulfuric acid per acre), and 4.9, (two tons of sulfuric acid per acre). No test for soluble iron was obtained. Phosphorus was slightly more soluble at a pH of 4.2 than at any other except at pH 7.2 which, as mentioned before, may be due to the lack of a clear filtrate. This soil was very low in potash. The check contained 7 p.p.m. Potash became most soluble with the addition of the first increment of sulfuric acid. Successive increments had little effect on the solubility of potash. Lime additions did not increase the solubility of potash.

Soil V is the highest soil of the six samples in organic carbon content, 2.35 per cent, (Table 7) and in total nitrogen content, 0.270

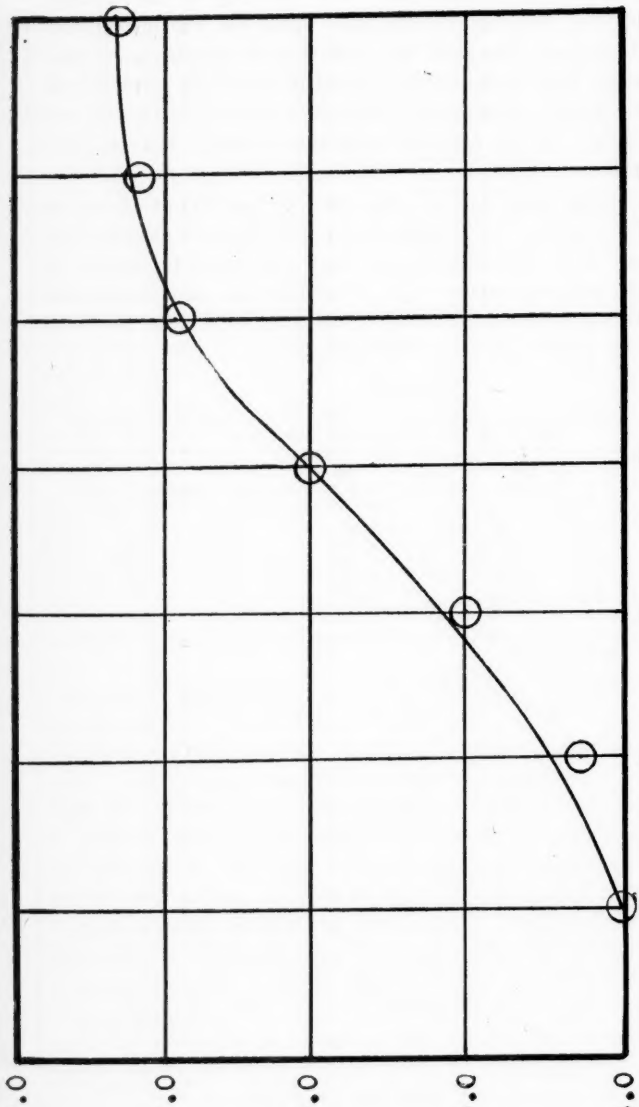

FIG. 4. Buffer Curve for Toa silt loam, IV

per cent. It is a highly buffered soil, being resistant to changes in reaction near neutrality. Although very low in potash the solubility of this nutrient element increased somewhat with the addition of one ton of sulfuric acid per acre. Lime additions did not affect the solubility of potash. The solubility of phosphorus was not increased markedly on either side and was comparatively low in this soil.

TABLE 6

RELATION OF CHANGE IN SOLUBILITY OF NUTRIENT ELEMENTS TO CHANGE IN REACTION IN TOA SILT LOAM, VI

Treatment	Ca p. p. m. *	Mg p. p. m.	Mn p. p. m.	Fe p. p. m.	P p. p. m.	K p. p. m.	pH
1	625	115	4	no test	1.1	100	4.3
2	555	135	no test	no test	1.2	30	4.8
3	370	135	no test	no test	1.5	30	6.1
4	190	148	no test	no test	1.9	10	6.9
5	355	115	no test	no test	1.9	60	7.2
6	355	148	no test	no test	1.9	none	7.4
7	500	135	no test	no test	2.0	none	7.8

* On the basis of dry soil.

Table 6 shows that Soil VI responded markedly to both additions of sulfuric acid and lime, as shown by the increase in solubility of the lime content. The solubility of magnesium was changed only slightly from additions of sulfuric acid and lime. Soil VI is low in manganese. Manganese was insoluble above pH 4.8. Phosphorus was less soluble on the acid side. Additions of lime did not increase the solubility of phosphorus. Soil VI is low in soluble potash, but the additions of acid brought a marked increase in its solubility. The addition of one ton of lime per acre increased the solubility of potash but with the two and three ton increments the solubility of potash became too small to be measured. The reason for the increase from the first increment of lime may have been due to the stimulation of extra organic decomposition during the period of one week between the treatment and drying of the soil.

The organic carbon and nitrogen contents, 1.66 and 0.205 per cent respectively, show that this soil is intermediate in organic matter content and the buffer curves show that the soil is not so well buffered near neutrality as Soils I and V which contain more organic matter. However, Soil VI is more highly buffered near neutrality than Soils II and III whose buffer curves are of the sigmoid type which is characteristic of the soils low in organic matter.

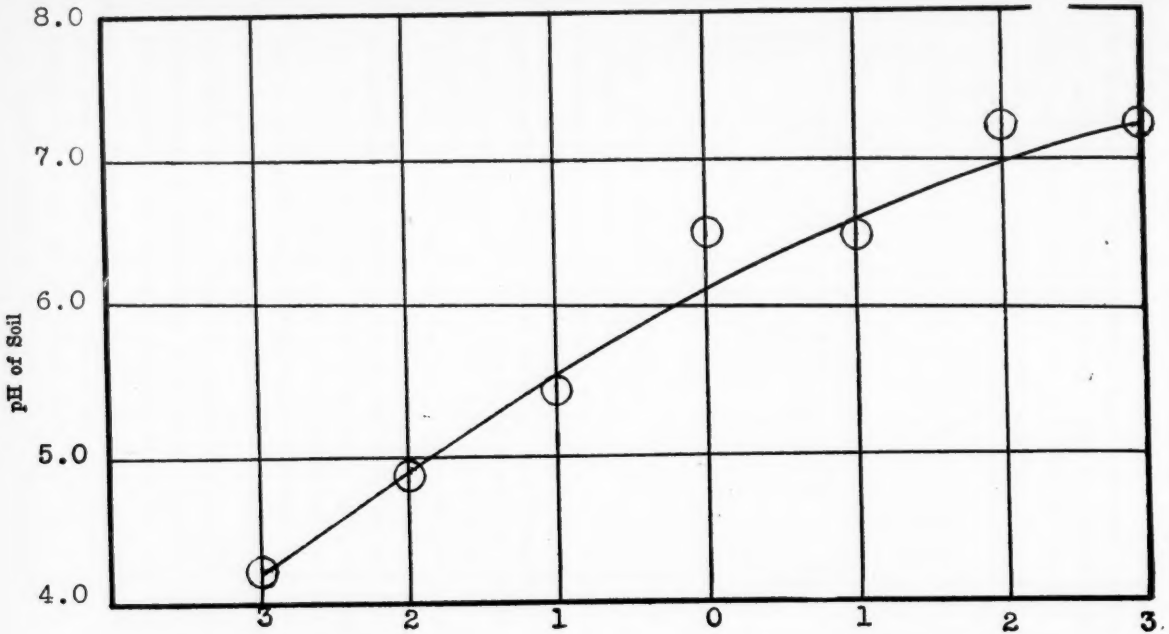

FIG. 5. Buffer Curve for Toa silt loam, V

TABLE 7

THE ORGANIC CARBON AND NITROGEN CONTENTS AND RATIOS IN THE SIX SAMPLES OF TOA SILT LOAM STUDIED

No. of Soil	% Moisture	% Organic carbon	% Total N	C/N
I	1.55	2.25	0.235	9.5
II	0.79	1.19	0.126	9.4
III	1.15	1.44	0.150	9.6
IV	1.31	1.61	0.199	8.1
V	1.03	2.35	0.270	8.7
VI	0.69	1.66	0.205	8.1

It can be seen from Table 7 that the organic matter in the six samples of soil is well decomposed or largely in the humic state. This is indicated from the relatively narrow carbon-nitrogen ratios. From this it can be inferred that the changes in the buffering effect as related to the different amounts of organic matter in the samples is largely controlled by the lignin-humus fraction of the organic matter (10, 11) which makes up the major part of it.

SUMMARY

1. Organic matter, buffer capacity, and the relation of changes in reaction to the solubilities of the mineral nutrient elements in six samples of Toa silt loam were studied.

2. The buffer characteristics may vary rather widely within a given soil type. The buffer curves for different samples of Toa silt loam show that the presence of larger amounts of organic matter markedly increases the buffer capacity near neutrality. This is reflected in the flatter buffer curves. The samples which contained lower quantities of organic matter are most highly buffered at the extreme ranges of high acidity and basicity. This is indicated by typical sigmoid buffer curves.

3. The state of the decomposition of the organic matter in these samples indicates as has been shown by previous work that the buffering effect of the organic matter is associated with the lignin-humus fraction.

4. Additions of acid to the soil increased the solubility of the nutrient mineral elements. The increases in the solubilities were greatest in the samples that were low in organic matter.

5. Although liming increased the amount of soluble calcium in the soil, it did not decrease the solubility of phosphorus. The additions of lime decreased the solubility of potassium. The solubilities of iron and manganese were too low to be easily measured above pH 7.2 and in only one case above pH 5.0.

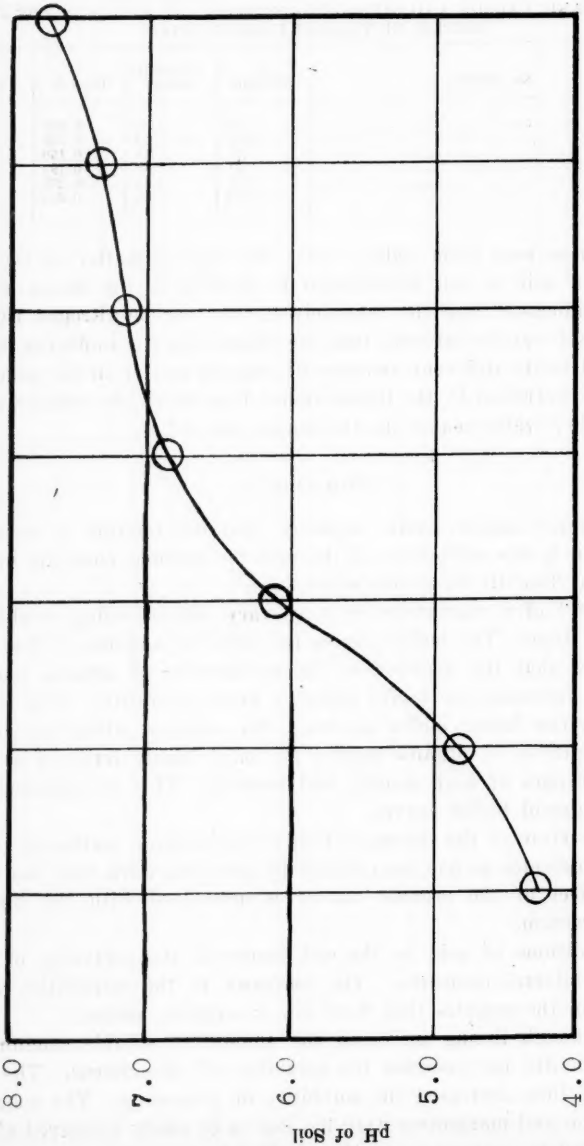

FIG. 6. Buffer Curve for Toa silt loam, VI

6. Additions of acid to the soil increased the solubility of potassium when the reaction was forced lower than pH 5.0. There was a greater increase in the solubility of potassium from the acid treatments in the samples that were lower in organic matter.

7. The solubilities of iron and manganese were increased from additions of acid. Manganese became much more soluble than iron from increased acidity and its solubility was more affected in the samples that were low in organic matter.

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FIG. 6. Buffer Curve for Toa silt loam, VI

CaCO$_3$ per Acre

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